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The biological basis for the use of protein growth stimulant made from cattle split for wheat foliar feeding and disease suppression

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Abstract. The new modern preparation – protein growth stimulant – was generated in accordance with technology of employees of Saint-Petersburg ITMO University and Saint-Petersburg State Agrarian University. Biological activity of the preparation was determined by measurements of 20 indicators of the wheat productivity. In addition, 16 indicators of different types of pathogenesis were determined. These can be formed at distribution of *Helminthosporium* root rot, wheat rust species, powdery mildew and wheat leaf blotch. The use of the protein growth stimulant promoted increase of potential yield in 80% of samples. In comparison with the control, 15 wheat varieties, treated with the preparation, showed an increase in the main productivity indicators: the length of the spike, the number of spikelets per spike, the weight of 1,000 grains, the productive tilling capacity and the general bushiness. The intensity of *Helminthosporium* root rot development decreased 11.9% (it was found in 53.3% of samples) and the wheat leaf blotch by 15.6% (in 66.7% of samples). The wheat brown rust development intensity decreased insignificantly (3.6% compared to the control). In the same time, values of the pathogen pustule area decreased at average by 79.8%. There was an increase in total nitrogen in wheat leaves at 92% of samples. As was revealed, the effectiveness of the protein growth stimulant largely depends on the wheat variety. To conclude, the prospect of using the new effective protein growth stimulant to increase productivity of wheat and protection from diseases was shown.

Key words: diseases of wheat, growth stimulants, soft wheat, the elements of productivity.

INTRODUCTION

Environmental safety of food production is one of the main requirements for agricultural products of modern world agroindustrial sector. Intensive nature management with its chemicalization and receipts of toxic substances in agrocenoses causes degradation of soil cover and destruction of microflora. All these factors lead to an increased content of toxic substances and compounds in crops of cultivated plants. Russia, along with many economically developed countries, revises the concept of agricultural production development, shifts it in the direction of reducing the external anthropogenic impact on agrocenoses and for creating an enabling environment for the realization of their own potential (Pavlyushin & Lysov, 2019). Organic agriculture is one of the forms for realization of this task. It assumes application of natural growth stimulants as inducers of plant resistance to diseases (Čepulienė & Jodaugienė, 2018; Kolesnikov et al., 2018; Siah et al., 2018; Khan et al., 2019) and yield increase.

Active study of the effect of amino acid fertilizing on plants begun in the 70–80´s of the last century and it continues to the present (Che et al., 2011; Sudisha et al., 2011; Hammad & Ali, 2014). Many scientists have noted that amino acids activate the mechanisms of plant growth after exposure to stress and low temperature (Soro, 1985), improve the pollen fertility and the formation of fruit ovaries (Marcucci, 1984), increase the ability of assimilation of nutrients (Stoyanov, 1981), crop yield (Gulewicz et al., 1997) and pest and disease resistance (Kovacs et al., 1986; Wang et al., 2019). Biostimulants (amino acids and yeast) have a positive effect on most of the physiological signs and wheat crop yield elements, if wheat has experienced water stress during drought. This contributes to overcoming the harmful effects of high temperatures on crops (Hammad & Ali, 2014).

A number of studies have found that plants are able to quickly and better absorb natural α-amino acids (from which proteins are built) of optically active L-configuration. L-α-amino acids are easily absorbed by plants and quickly incorporated into the metabolism as their own (Kretovich, 1986). Glutamate is a universal amino acid and is central to both mammals and higher plants. As a result of post–harvest treatment of pear fruits with L–glutamate (L-Glu), there was a significant inhibition of the development of blue mold on them caused by *Penicillium expansum Link ex. Thom.* at different storage temperatures (Jin et al., 2019). The combined use of L–glutamate (or glycine) complexes with copper-containing bactericides reduced the side toxic effects of copper on plants (Niu et al., 2018). Biochemists have found that unsaturated amino acids (UnsAA (BCAAs)) affect the activity of various biological processes. However, the mechanisms of their action on plant cells are poorly understood, so their research is currently underway (Roblin et al., 2016).

In Russia, such amino-containing preparations are registered as an anti-stress agent – Megaphol; stimulator of harmonious development- Viva, and stimulator of root formation- Radipharm (Valagro). Despite the effectiveness of the action, their production and application did not have a large-scale use in crop production due to economic reasons. Currently, a promising direction is the creation of new domestic biological plant growth stimulants obtained by chemical hydrolysis from by-products of slaughter animals (Kutsakova et al., 2013, RF patent No. 2533037). When using this stimulant (hereinafter named as growth stimulant) on fruit and berry crops, their earlier maturation, increase in productivity and improving the quality of fruit and strengthening the protective mechanisms of phytoimmunity was noted (Kremenevskaya et al., 2017). The preparation is undergoing for active testing in the School of Biotechnology and Cryogenic Systems, ITMO University at the Department of Plant protection and quarantine of Saint-Petersburg State Agrarian University. It is derived from a by-product of cattle processing (list of cattle shins). The mechanism of action of amino acids containing preparations assumes their good digestibility by plants and absence of phytotoxicity. Glutamic acid, cysteine, glycine, histidine, lysine are included in the complex form of chelated compounds with trace elements. Tyrosine, arginine, alanine, proline, serine, threonine and valine in its composition stimulate plant metabolism and enhance the adaptive potential of plants to environmental factors (Bityuckij, 1999).

The aim of the study wass to substantiate the prospects of using the protein growth stimulant from a by-product of cattle processing to increase the yield and quality of grain and to reduce the injuriousness of wheat pathogens.

MATERIALS AND METHODS

Production of the protein growth stimulator

Canned beef split was used to receive the protein hydrolysate as the stimulator. Hydrolysis was carried out according to the research methods described by Kremenevskaya et al., 2017. The composition of raw materials was always monitored to avoid possible negative divergences and their impact on the finished product. The hydrolysate was dried by the use of the semi-industrial unit (Kutsakova, 2004). Studies of the amino acid composition of the stimulant were carried out in two-dimensional liquid chromatography system using Nexera-e software (Shimadzu Corporation, Japan) in the Leningradskaya inter-regional veterinary laboratory of feed and grain safety and quality. The moisture content of the samples was calculated, using the universal humidity analyzer MX-50 (AND, Japan); the sodium chloride concentration was measured using the PAL-SALT Mohr salinometer (ATAGO, Japan), and the pH and calcium ion content were measured using the I – 510 microprocessor ionomer (Aquilon company, Russia).

Plant material and location of the experiment

Experimental research was carried out in the experimental field of Pushkin laboratories of N.I. Vavilov Federal research Center of the all-Russian Institute of plant genetic resources (VIR). The field experiment to determine the effect of protein growth stimulant on the productivity and intensity of wheat diseases was performed on 15 samples of wheat of different origin: LP–588–1–06, c–65446 (catalog number) (Germany); Uralosibirskaya, c – 65244 (Russia, Omsk region); c–32666 (Russia, Sverdlovsk region); Orenburgskaya 22, i–147624 (Russia, Orenburg region); Pamyati Judina, c–65243 (Russia, Irkutsk region); Leningradskaya 97, c–62935 (Russia, Leningrad region); Amurskaya Krasnokoloska, c–32095 (Russia, Amur region); Ulianovskaya 100, c–65250 (Russia, Ulyanovsk region); Kampanin, c–65445 (Germany); Vasilisa, c–65443 (Belarus); Tyumenskaya 30, c–65248 (Russia, Tyumen region); Krasnouphimskaya 110, c–65478; Tuliaykovskaya 108, c–65452 (Russia, Samara region); Tuliaykovskaya 110, c–65454 (Russia, Samara region); Tyumenskaya 29, c–65247 (Russia, Tyumen region). These samples were provided for study by the VIR wheat genetic resources Department.

Treatment of plants with the protein stimulant and the wheat productivity analysis

Prophylactic spraying of wheat with a growth stimulant was carried out in three-fold repetition: the phases of tillering, stem elongation, and beginning of ear formation.

The concentration of the aqueous solution of the stimulant was 195 mg L^{-1}. The phases of plant development were determined by the generally accepted Zadoks scale (Zadoks et al., 1974). Wheat productivity was studied in three-fold repetition in the phase of earing-flowering and maturation by a set of indicators characterizing the morphological characteristics of plants and the structure of yield. In the earing–flowering phase, a complex of plant indicators was studied: productive and general bushiness (pieces), plant phase (digital code, Zadoks scale), flag and pre-flag leaf area (cm^{-2}), height of plants (cm) length of spike (cm), number of spikelets in the spike (piece), weight of spike (g). In addition, the number and length of roots (the main embryonic root, embryonic coleoptile and roots) extending from the epicotyl was determined. The indices of root mass and vegetative part of plants were calculated. The sample size for each variant of the experiment was 10–15 plants, which provided a reliable result for measuring the complex of the indicators at $P > 0.95$.

In the maturation phase (phase of full ripeness), the structure of the following wheat yield indicators was studied: number of the spikelets per spike; the spike length, cm; the spike weight; the grain weight per spike; the number of grains per spike; and the 1,000 grains weight. The potential biological yield of a single wheat plant (g $plant^{-1}$) was estimated in accordance with the values of the productive bushiness of one plant and the grain weight per spike. The potential yield (Y_p) of wheat varieties in relation to the area of sowing (t ha^{-1}) was calculated by the productive bushiness, the grain weight per spike, and the number of plants sown per one m^2 according to the original formula:

$$Y_p = M_k \cdot К_p \cdot P_p \cdot 10{,}000 \quad (1)$$

where M_k – grain weight per spike (t); K_p – productive bushiness of the sample; P_p – sowing density (number of plants per one m^2).

In estimation the grain weight per spike and productive bushiness for each variant of the experiment, the amount of sampling was 10–15 plants.

The total nitrogen content in the flag leaves of soft wheat was calculated at the beginning of flowering stage using of the photoelectrometric method (Ermakov, 1987) in accordance with the GOST 10846-91.

Methods for assessing the intensity of wheat root rot

Assessment of the degree of damage to plants by root rot was carried out in laboratory conditions in the phase when wheat finished tillering and earing- lowering according to the scale: 0 – epicotyl without lesion, 1 – single spots on epicotyl; 2 – strong defeat; 3 – fatal defeat, a plant is died. In each variant of the experiment, 20 plants were evaluated. The development of root rot according to the variants of the experiment was determined, taking into account the weighted average of the degree of plant damage (Popov, 2011):

$$R_r = \frac{\sum(\mathrm{a} \cdot b) \cdot 100}{A \cdot K} \quad (2)$$

where R_r – development of root rot; a – number of plants with the same signs of damage; b – the corresponding score; A – number of plants registered (healthy and sick); K – the highest score of the accounting scale.

From each experimental plot, 20 plants with the roots were dug out randomly. Underground plant parts were carefully removed from the soil and then washed in running water. The main germinal roots, germinal and coleoptile roots, and nodal roots were visually analyzed. The root rot development was evaluated in a natural infectious background. The main causative agent of the disease was *Bipolaris sorokiniana (Sacc.) Shoem*.

Methods for assessing the intensity of wheat foliar diseases

The development intensity of the pathogens of wheat leaves was taken into account in the following phases and stages of wheat ontogenesis: the phase of wheat tillering (finished tillering), the phase of flag leaf (the leaf-tube formation, the last leaf sheath opening), the phase of earings (the end of the earings), phase of flowerings (the start of flowerings and the end of the flowering), maturation (milk ripeness of grain; wax ripeness). The intensity of the flag and pre-flag wheat leaves defeat by the powdery mildew (*Blumeria graminis* Speer.) was assessed visually according to the degree of plant damage (Fig. 1), as well as additional indicators – the number and area of powdery mildew stains.

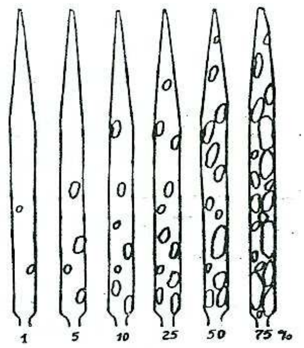

Figure 1. The account scale for the intensity of cereals defeat by the powdery mildew.

The number of powdery mildew stains was determined by counting them, based on the area of the wheat leaf: $S_п = 0.7 \cdot L \cdot S$, (where 0.7 is the narrowing factor, L – length of leaf (cm), S – width of leaf (cm)). When a large number of spots with a touch of powdery mildew on the leaf was registered, their number was first counted in one cm^2 of the leaf, and then it was transferred to the value to the entire area of the wheat leaf. For each variant of the experiment, the values of the above indicators of the wheat powdery mildew pathogenesis were determined by the results of the analysis of 15 flag and 15 pre-flag leaves.

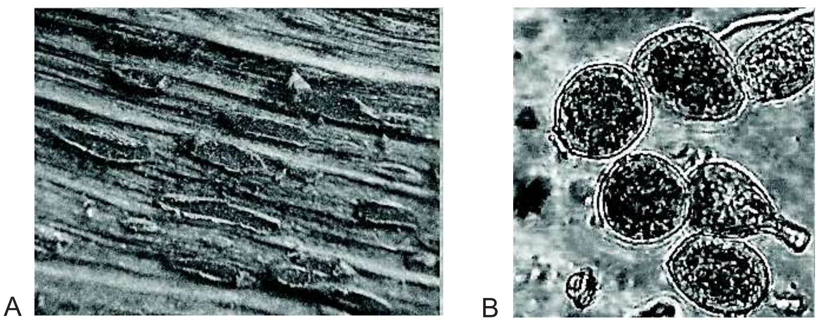

Figure 2. Symptoms of wheat brown rust on the soft wheat leaves (A – uredospore pustules arranged on the flag leaf randomly, magnification of 16^x; B – spherical uredospores, magnification of 800^x).

Defeat of wheat flag and pre-flag leaves by brown rust *Puccinia recondita Rob. ex Desm. f. sp. tritici Eriks.* (Fig. 2) was counted on the Peterson scale (Fig. 3). The number of pustules per leaf and the area of the pustule were used as an additional phytopathological parameters. The number of pustules was determined by counting them on wheat leaves using the MBS–10 microscope (Lytkarino Optical Glass Factory, Russia).

The degree of sample damage by wheat leaf blotch (*Stagonospora nodorum Castell.et Germano*) was determined according to the visual scale of James (James, 1971). For each experimental variant, the intensity of leaf blotch development (Fig. 4) was characterized by the results of the analysis of 15 flag and 15 pre-flag leaves.

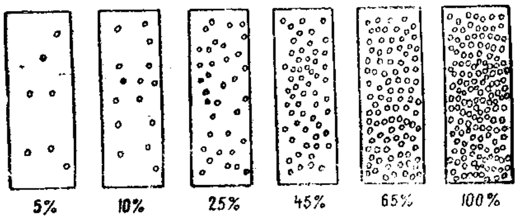

Figure 3. The Peterson account scale for the plant defeat by the brown rust (Peterson et al, 1948; The methods…,1988).

A

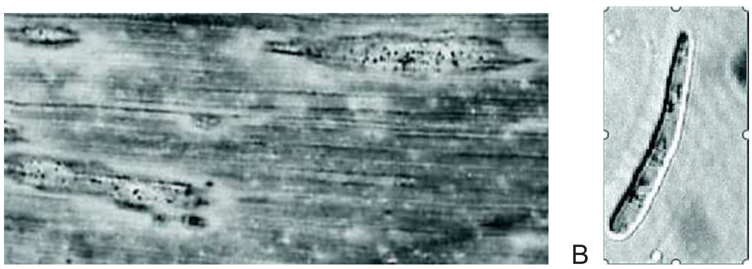

Figure 4. Symptoms of wheat leaf blotch on the soft wheat leaves (A – spots with pycnidia, magnification of 16^x; B – pycnidiospore, magnification of 800^x).

The intensity of wheat defeat by yellow rust (*Puccinia striiformis* West. (*Puccinia glumarum* Eriks. et Henn.) (Fig. 5) was assessed on the Manners scale (Fig. 6); and, in addition, visually, with using a microscope MBS-10 (Lytkarino Optical Glass Plant, Russia). The number of pustules (total per leaf), number of yellow rust stripes with pustules, the length of stripes with pustules, the area of the pustule and their number in the stripe were used as an additional indicators of pathogenesis.

A

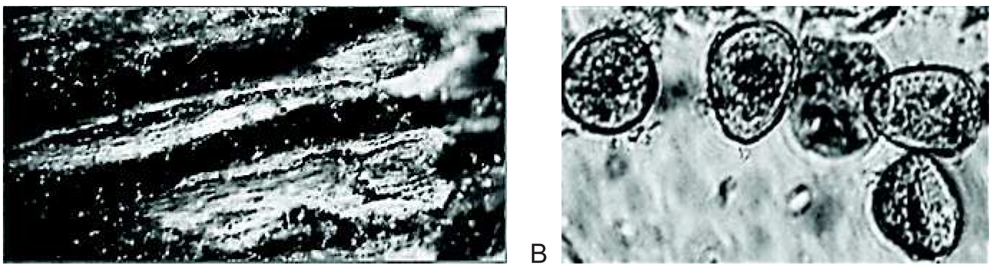

Figure 5. The symptoms of wheat yellow rust on the leaves of soft wheat (A – uredospore pustules in strips on the flag leaf, magnification of 56^x; B – rounded uredospores, magnification of 800^x).

For each variant of the experiment, the values of the above indicators of yellow rust pathogenesis were determined by the results of the analysis of 15 flag and 15 pre-flag leaves.

The size of the powdery mildew stains and rust's pustules (brown & yellow rust), which were formed in pathogenesis on wheat leaves, was determined using eyepiece and stage micrometers.

The values of pustule area and powdery mildew stains (infectious structures $S_{i.s.}$ were calculated under the assumption on their elliptic form using the expression:

$$S_{i.s.} = m \cdot \pi \cdot a \cdot b \qquad (3)$$

where a and b – the values of the semiaxes of the ellipse (in the lines of the eyepiece micrometer), m – the scale factor of the microscope.

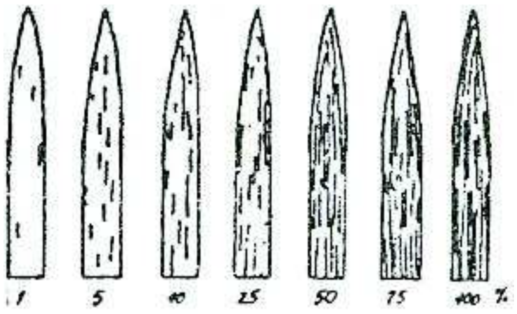

Figure 6. The Manners account scale for the degree of plant defeat by the yellow rust (Babayantc et al., 1988).

The use of this complex of pathogenesis indicators allowed to expand the range of statistical analysis methods applicable to the study and to increase the accuracy of the experiment in determining the biological effectiveness of a protein growth stimulant. Statistical analysis of the results was carried out in the programs SPSS 21.0, Statistica 6.0 and Excel 2016. In the calculations, the methods of parametric statistics were used (based on *mean* and their *standard errors ±SE*; and *95% confidence intervals* and the *Student's t-test*).

RESULTS AND DISCUSSION

Effect of the protein growth stimulant on the wheat yield

The main active ingredient in the preparation, which has a stimulating effect, is the aminoacid glycine (1.20 μmol mg^{-1}). Other significant amino acids in the stimulant were: proline (0.63 μmol mg^{-1}), alanine (0.58 μmol mg^{-1}), glutamic acid (0.41 μmol mg^{-1}) and aspartic acid (0.155 μmol mg^{-1}). The concentration of the aqueous solution of the stimulant was 0.195 g L^{-1}.

At the first stage of the study, the comparison of wheat productivity indicators in the variants of the experiment was carried out: when treated with a protein growth stimulant and without treatment (control group). Growth stimulant increased the yield in 80% of samples (Fig. 7). Compared with the control (untreated samples), growth stimulator had the greatest effect on the biological yield of five wheat varieties: Tuliaykovskaya 108, c–65452 (103%, t = 2.6); Pamyati Judina, c–65243 (86.8%, t = 2.8); Krasnouphimskaya 110, c–65478 (77.9%, t = 2.9); Uralosibirskaya, c – 65244 (56.4%, t = 3.1); Orenburgskaya 22, i–147624 (43.2%, t = 2.0), where t is the *Student's criterion*.

It should be noted that highly productive varieties take out a large amount of nutrients from the soil and consume a lot of water. Therefore, such varieties require a high level of agricultural technology. If there are no such conditions, a potentially more productive variety not only does not give a yield increase, but can also lose to another variety, less productive, but also less demanding to the cultivation conditions.

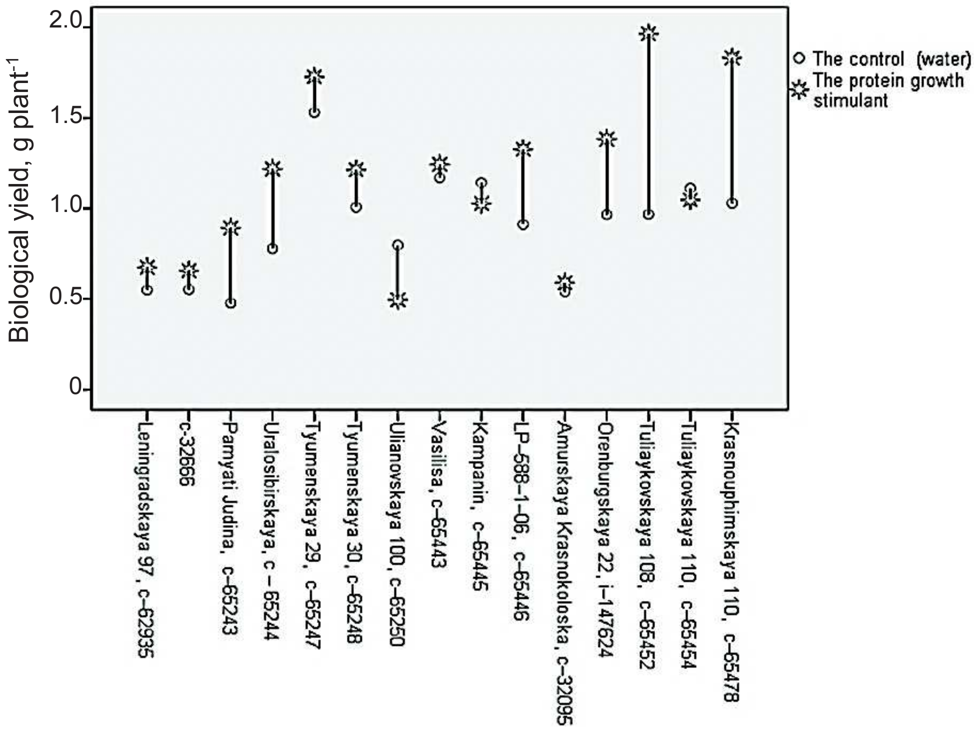

Figure 7. Changes in the wheat biological yield when using the protein growth stimulator.

If the growth stimulant was used, the increase of the main indicators of wheat productivity was noted as follows:

- productive bushiness – 15.4% (found in 46.7% of samples, but the increase in the values of the indicator was not statistically significant);
- total bushiness – 22.5% (revealed in 60.0% of samples, statistically significant in 26.7% of samples);
- spike length – 17.9% (revealed in 73.3% of samples, statistically significant in 46.7% of samples);
- the number of spikelets per spike – 16.1% (revealed in 73.3% of samples, statistically significant in 46.7% of samples);
- weight of 1,000 grains – 11.5% (73.3% of samples, statistically significant in 33.3% of samples);
- spike weight – 32.9% (found in 66.7% of samples, statistically significant in 40.0% of samples).

General and productive bushiness usually makes up for density in the field and it is a useful biological adaptation of plants to environmental conditions. When using a protein growth stimulant, its predominant effect on the overall bushiness of plants was revealed. The most important indicators that directly determine the wheat yield are the spike length, the number of spikelets per spike, the 1,000 grains weight and the spike weight. To the greatest extent, the plant growth stimulant had an impact on the growth of the spike length and the number of spikelets per spike. In the analysis of positive and reliably positive changes (according to the *Student's test* at $P < 0.05$), protein growth stimulant exerted the most effect on the spike weight, the 1,000 grains weight, the number of grains per spike, the number of spikelets per spike, the spike length and the total tillering (Fig. 8).

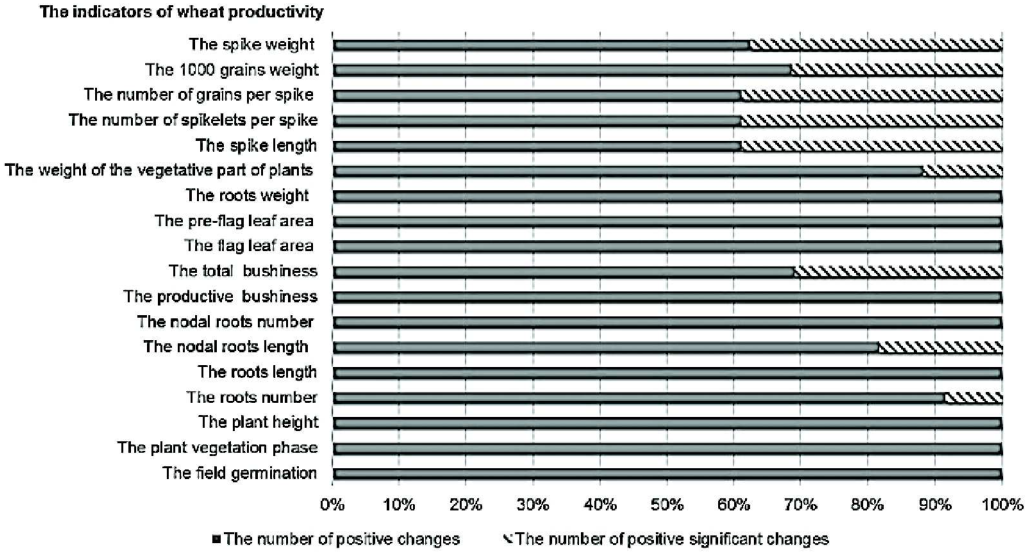

Figure 8. The number of changes in the wheat productivity indicators values when using the protein growth stimulator compared to the control (%).

The growth of the wheat flag leaf area directly correlates with the increase in photosynthetic activity of the plant and determines the increase in the yield and is also related to the spike length and the number of spikelets per spike. In addition, the growth stimulant caused the acceleration of plant development in the ontogenesis phases (by 5.9% in 66.7% of samples); increase in plant height (11.5% in 86.7% of samples), flag leaf area (17.4% in 60.0% of samples), pre-flag leaf area (8.9% in 33.3% of samples), and weight of roots (by 34.9% in 80.0% of samples). Wheat resistance to lodging is directly related to the height of plants. However, short-stemmed wheat forms suffer from drought and, in particular, they are not adapted to cultivation in continental conditions of some regions of Russia, for example Western Siberia.

It should be noted that the total N content in the flag leaves during the period of flowering is able to have significant influence on yield of wheat. In the number of experiments, a close positive relationship was observed between the grain protein content and N content of the flag leaves, especially at nitrogen evaluation in the flowering – the beginning of grain formation. A close negative correlation was revealed between the concentration of amino acids in wheat leaf juice and the concentration of gliadins and glutenins; a close positive correlation was found between the concentration of amino acids in leaf juice and the content of water-soluble proteins, globulins, non-extractable proteins, as well as the acid and alkaline proteases activity (Novikov, 2017). Significantly, foliar treatment with the protein growth stimulant caused an increase in the total N in wheat leaves in 92% of samples by an average of 84.6%.

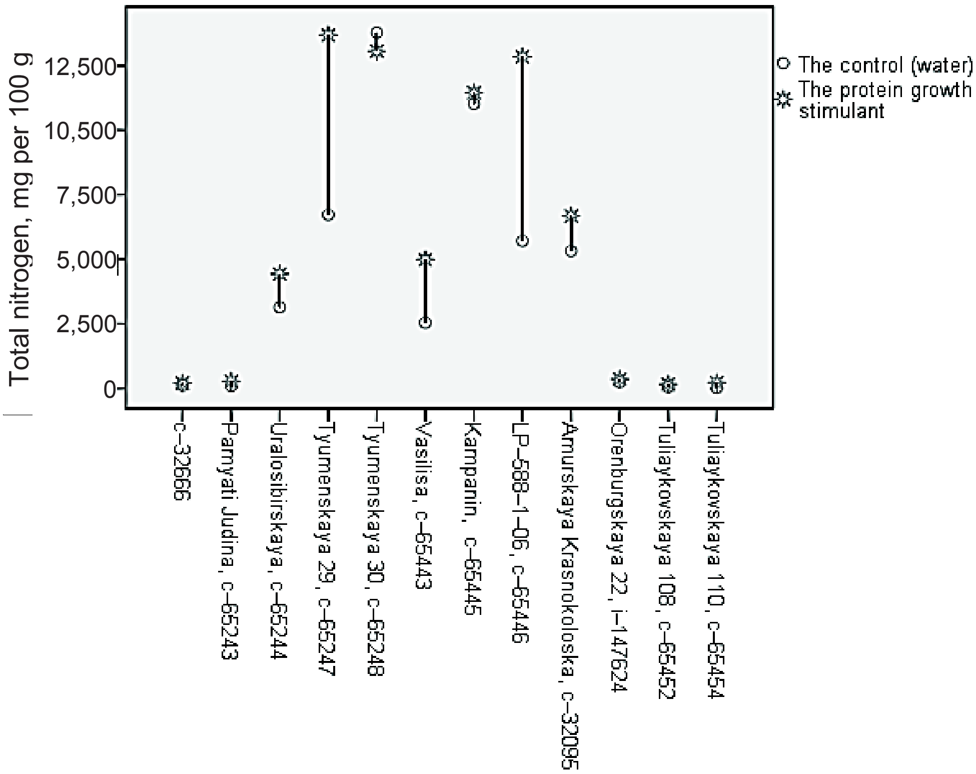

Figure 9. Changes of the total nitrogen content in the flag leaves at the beginning of wheat flowering when using the protein growth stimulator.

Effect of the protein growth stimulant on suppression of wheat diseases

In the second stage of the study, the effect of protein growth stimulant on the intensity of wheat diseases was studied. The use of the stimulator showed that the intensity of *Helminthosporium* root rot decreased by 11.9% in 53.3% of the samples. To the greatest extent (22%), a decrease in the development of the disease was observed on wheat cultivar Pamyati Judina, c–65243, and 17% on cultivar Tyumenskaya 29, c-65247. *Helminthosporium* rot of wheat belongs to the group of the most harmful diseases of ecological and parasitic nature, i.e. pathogen is present in the soil and affects weakened plants. It can be assumed that the use of the growth stimulant increased the adaptive potential of plants to external environmental factors, including this disease.

When plants were treated with the growth stimulant, there was a decrease in the wheat brown rust development intensity in 40% of the studied samples by 3.6%. In addition, use of growth stimulant caused a decrease in the values of the pustule area of wheat brown rust in 60% of the samples (an average at 79.8%) – the most important indicator of varieties to disease resistance. In this regard, it can be assumed that the protein stimulator of plant growth contributed to the activation of phytoimmunity reactions to the disease and after a number of additional experiments, it could be recommended as an inducer of wheat resistance to brown rust.

Use of growth stimulant decreased the intensity of wheat leaf blotch development on the pre-flag leaves 15.6%, compared to the control. This pattern was found in 66.7% of samples. The greatest degree of leaf blotch reduction with the use of a growth stimulant was registered in varieties Krasnouphimskaya 110, c–65478 (37.5%, $t = 3.0$) and Tuliaykovskaya 110, c–65454 (36%, $t = 7.9$). In recent years, wheat leaf blotch has been included in the list of especially dangerous diseases, in particular for wheat. This is the reason of emergency situations in crop production. With moderate development of the disease, crop losses can be 10–15%, when epiphytotics is 30–40%. One of the reasons for the ubiquitous wheat leaf blotch infection is the lack of varieties resistant to the disease. The use of the protein growth stimulant insignificantly reduced the disease development in more than half of the studied wheat varieties.

It should be noted, that the intensity of wheat infection by pathogens substantially affects the elements of productivity and the structure of wheat yield. The mathematical models describing the injuriousness of wheat pathogens, including in mixed infections, was previously developed by the authors.

According to the analysis of 250 soft wheat samples (Kolesnikova & Kolesnikov, 2012; Kolesnikov et al, 2012), it was found that the dependence of yield loss (by the grain weight per spike of one plant, %), Y_z from the brown rust pustule area $S_{b.p.}$, the numbers of pustules $N_{b.p.}$, and wheat flag leaf area $S_{l.z.}$ can be described by a multivariate linear regression equation: $Y_z = -134.654\ S_{b.p.} - 0.023\ N_{b.p.} - 0.021\ S_{l.z.}$ ($R^2 = 0.67$; $F = 7.67$; $P = 0.05$). The dependence of the wheat yield loss (by the grain weight per spike of one plant, %) from the number of pustules can be expressed by the equation: $Y_z = -0.219\ N_{b.p.}$ ($R^2 = 0.48$; $F = 52.03$; $P = 0.00$). The number of pustules of the pathogen $N_{b.p.}$ can be determined, based on the data of the conditional development of the disease R_b.: $N_{п.б.} = 3.931\ R_b$. According to the model, an increase in disease development by 1% (5 pustules per leaf) led to crop losses 1.1%; by 25% - to crop losses of 21.88%. The size of the pustule area, as a factor of wheat resistance to the disease, significantly affects the value of wheat yield loss Y_z (by the grain weight per spike, %). The dependence of Y_z on the brown rust pustule area S_{bp} can be expressed by the equation: $Y_z = -327{,}405\ S_{б.п}$ ($R^2 = 0{,}598$; $F = 19{,}36$; $P = 0.001$) (Kolesnikova & Kolesnikov, 2012).

A regression equation describing the relationship between the development of brown rust R_b, wheat leaf blotch R_s and yield loss per plant (by the number of grains per spike Yn.z. and by grain weight Yz.), has the form: $Y_{n.z} = -1.673R_б - 2.033R_s$ ($F = 13.81$, $P = 0.04$) and $Y_{z.} = -1.662R_б - 2.032R_s$ ($F = 9.04$, $P = 0.008$). An increase in the development of a diseases complex by 1% led to the following decrease in yield: $Y_{n.z} = 3.71\%$ and $Y_{z.} = 3.7\%$.

CONCLUSIONS

This study has shown the prospects of using the protein growth stimulant to increase productivity and protect wheat from diseases. However, the effectiveness of the growth stimulant depended heavily on the variety. When using the growth stimulator, a significant increase in the potential yield of wheat varieties of mainly Russian selection with a relatively high adaptive potential to the agroecological conditions of the Leningrad region was revealed. The maximum increase in potential yield (103%) compared to the control was registered on the variety Tuliaykovskaya 108, c–65452 (Russia, Samara region), which is determined by the maximum productivity of the variety in comparison with other cultivars. Reliable effect of the preparation was based on increase of the length of spike, number of spikelets per spike, number of grains per spike, weight of 1,000 grains. In addition, for this variety, foliar spraying of plants with a growth stimulant caused a significant decrease in the wheat leaf blotch intensity (36.3%). To the greatest extent, the growth stimulant caused a significant increase in the spike length, the number of grains per spike and the grain weight per spike on the cultivar Pamyati Judina, c–65243 (Russia, Irkutsk region) (44.3%, 66.7%, 68.9%, respectively); the number of spikelets per spike on the cultivar Tyumenskaya 30 c–65248 (21.2%); the 1000 grains weight on the cultivar Uralosibirskaya, c – 65244 (Russia, Omsk region) (22.9%); and the grain weight per spike on the cultivar Orenburgskaya 22, i–147624 (Russia, Orenburg region) (49.3%).

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REFERENCES

Babayanc, L, Meshterhazi, A., Vekhter, F., Neklesa, N., Dubinina, L., Omel'chenko, L., Klechkovskaya, E., Slyusarenko, A., Bartosh, P. 1988. *The methods of infection background creation and estimation of wheat and barley resistance to main diseases*. Prague, 322 pp.

Bityuckij, N.P. 1999. *Microelements and plant*. St. Petersburg state University publishing house, Saint-Petersburg, 156 pp. (in Russian).

Čepulienė, R. & Jodaugienė D. 2018. Influences of biological preparations on soil properties in the spring wheat crop. In Raupelienė, A. (ed): *Proceedings of the 8th International Scientific Conference Rural Development 2017.* Aleksandras Stulginskis University, Kaunas, pp. 31–36. http://doi.org/10.15544/RD.2017.013

Che, Y.Z., Li, Y.R., Zou, H.S., Zou, L.F., Zhang, B. & Chen, G.Y., 2011. A novel antimicrobial protein for plant protection consisting of a *Xanthomonas oryzae* harpin and active domains of cecropin A and melittin. *Microbial Biotechnology* **4**(6), 777–793. doi:10.1111/j.1751-7915.2011.00281.x

Gulewicz, K., Aniszewski, T. & Cwojdziński, W. 1997. Effects of some selected lupin biopreparations on the yields of winter wheat (*Triticum aestivum L.* ssp. *vulgare* Vill) and potato (*Solanum tuberosum L.*). *Industrial Crops and Products* **6**(1), 9–16. https://doi.org/10.1016/S0926–6690(96)00171–9

Ermakov, A.I. 1987. *Methods of biochemical research of plants*. Leningrad, Agropromizdat, 430 pp. (In Russian).
Hammad, S.A.R., &. Ali, O.A.M., 2014. Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Annals of Agricultural Sciences* **59**(1), 133–145. https://doi.org/10.1016/j.aoas.2014.06.018
James, W.C. 1971. An illustrated series of assessment for plant diseases preparation and usage. *Canadian Plant Disease Survey* **51**(2), 39–65.
Jin, L., Cai, Y., Sun C., Huang Y. & Yu T. 2019. Exogenous L–glutamate treatment could induce resistance against *Penicillium expansum* in pear fruit by activating defense–related proteins and amino acids metabolism. *Postharvest Biology and Technology* **150**, 148–157. https://doi.org/10.1016/j.postharvbio.2018.11.009
Khan, S., Basra S.M.A., Nawaz M., Hussain I. & Foidl, N. 2019. Combined application of moringa leaf extract and chemical growth–promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum L.*). *South African Journal of Botany*. Available online 21 March 2019. https://doi.org/10.1016/j.sajb.2019.01.007
Kolesnikov, L.E., Pavlova, M.N. & Rykhlova, K.V. 2012. Influence of causative agents of diseases of leaves on productivity and economic efficiency of cultivation of spring wheat. *Izvestiya Saint–Petersburg State Agrarian University* **29**, 19–23 (in Russian).
Kolesnikov, L.E., Novikova, I.I., Surin, V.G., Popova E.V., Priyatkin, N.S. & Kolesnikova, Yu.R. 2018. Estimation of the efficiency of the combined application of chitosan and microbial antagonists for the protection of spring soft wheat from diseases by spectrometric analysis. *Applied Biochemistry and Microbiology* **54(**5), 546–552 (in Russian).
Kolesnikova, Y.R. & Kolesnikov, L.E. 2012. The productivity of spring soft wheat and its limitation by leaf disease agents. *Izvestiya Saint–Petersburg State Agrarian University* **27**, 60–67 (in Russian).
Kovacs, A.I., Maini, P. & De Leonardis, A. 1986. Nematostatic effect of biostimulant Siapton. In *Atti Giornate Fitopatologia*, Clueb Ed. Bo, Riva del Garda, pp. 415–424 (in Italian).
Kremenevskaya, M., Sosnina, O., Semenova, A., Udina, I. & Glazova, A. 2017. Meat industry by–products for berry crops and food production quality improvement. *Agronomy Research* **15**(S2), 1330–1347.
Kretovich, V.L. 1986. *Plant Biochemistry*. Higher school, Moscow, 503 pp.
Kutsakova, V.E. 2004. Drying of liquid and pasty products in a modified spouted bed of inert particles. *Drying Technology* **22**(10), 2343–2350.
Kutsakova, V.E., Frolov, S.V., Kremenevskaya, M.I. & Marchenko, V.I. 2013. Patent 2533037 RF: MPK C05 F 1/00, A 01 N 33/00. A method for producing a protein stimulator of plant growth and development. № 201334879/13; appl. 24.08.13; publ. 20.11.2014. – Bull. No. 32, 6 pp.
Marcucci, M.C. 1984. The influence of storage and of organic nutrients on the germination of pollen and fruit set of apple and pear. *Acta Horticulturae*: *Flowering and Fruit Set in Fruit Trees* **149**, 117–122.
Niu, J., Guo, D., Zhang, W., Tang, J., Tang, G., Yang, J., Wang, W., Huo, H., Jiang, N. & Cao, Y. 2018. Preparation and characterization of nanosilica copper (II) complexes of amino acids. *Journal of Hazardous Materials* **358**, 207–215. https://doi.org/10.1016/j.jhazmat.2018.06.067
Novikov, N.N. 2017. The new method for diagnostics of nitrogen nutrition and forecasting the quality of wheat grain. *Izvestiya TSHA*. **5**, 29–40 (in Russian).
Pavlyushin, V.A. & Lysov, A.K. 2019. Phytosanitary safety of agro–ecological systems and remote phytosanitary monitoring in plant protection. *Sovremennye problemy distantsionnogo zondirovaniya zemli iz kosmosa* **16**(3), 69–78 (in Russian).

Peterson, R.F., Campbell, A.B. & Hannah, A.E. 1948. A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. *Canadian Journal of Research Section C* **26**, 496–500. doi:10.1139/cjr48c-033

Popov, Y.V. 2011. Method for the estimation of the root rots development in cereals. *Plant protection and quarantine* **8**, 45–47 (in Russian).

Roblin, G., Laduranty, J., Bonmort, J., Aidene, M. & Chollet, J.F. 2016. Unsaturated amino acids derived from isoleucine trigger early membrane effects on plant cells. *Plant Physiology and Biochemistry* **107**, 67–74. https://doi.org/10.1016/j.plaphy.2016.05.025

Siah A. Magnin–Robert M., Randoux B., Choma C., Rivière C., Halama P. & Reignault P. 2018. Natural agents inducing plant resistance against pests and diseases. *Sustainable Development and Biodiversity. Natural Antimicrobial Agents* **19**, 121–159.

Soro, R. 1985. Stimulation of orange trees. *Agricola Vergel* **4**, 166–169 (in Spanish).

Stoyanov, I. 1981. Restoration of maize plants after Magnesium starvation with the help of Magnesium and Siapton. In *Proceeding of 3rd International Symposium on Plant Growth Regulators*, Varna, Bulgaria, pp. 602–606.

Sudisha, J., Kumar, A., Amruthesh, K.N., Niranjana, S. R. & Shetty, H.S. 2011. Elicitation of resistance and defense related enzymes by raw cow milk and amino acids in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. *Crop Protection* **30**(7), 794–801. https://doi.org/10.1016/j.cropro.2011.02.010

Wang, S., Bao, L., Song, D., Wang, J., Cao, X. & Ke, S. 2019. Amino acid–oriented poly–substituted heterocyclic tetramic acid derivatives as potential antifungal agents. *European Journal of Medicinal Chemistry* **179**, 567–575. https://doi.org/10.1016/j.ejmech.2019.06.078

Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A Decimal Code for the Growth Stages of Cereals. *Weed Research* **14**, 415–421.